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Regulation of Body Size and Behavioral State of *C. elegans* by Sensory Perception and the EGL-4 cGMP-Dependent Protein Kinase

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Summary

The growth and behavior of higher organisms depend on the accurate perception and integration of sensory stimuli by the nervous system. We show that defects in sensory perception in *C. elegans* result in abnormalities in the growth of the animal and in the expression of alternative behavioral states. Our analysis suggests that sensory neurons modulate neural or neuroendocrine functions, regulating both bodily growth and behavioral state. We identify genes likely to be required for these functions downstream of sensory inputs. Here, we characterize one of these genes as *egl-4*, which we show encodes a cGMP-dependent protein kinase. We demonstrate that this cGMP-dependent kinase functions in neurons of *C. elegans* to regulate multiple developmental and behavioral processes including the orchestrated growth of the animal and the expression of particular behavioral states.

Introduction

The perception of sensory cues allows an animal to respond appropriately to its environment and to adapt to changing conditions. Many sensory cues take the form of discrete stimuli that elicit attraction or avoidance behaviors. For instance, animals withdraw from noxious tactile or olfactory stimuli. In such instances, the properties of the stimulus largely account for the properties of the behavior. Sensory perceptions can also trigger or modify more complex patterns of behavior that are not consistently predicted by a specific stimulus. For example, in mammalian systems, appetitive behavior, aggression, and sexual behaviors are more variable in their expression. The variability in expression of such behaviors is often related to changes in the internal motivational state of organisms. The expression of these behaviors may reflect interactions between external stimuli and an internal state governed by endocrine factors, the autonomic nervous system, and other homeostatic processes. Perception of environmental stimuli can also result in adaptation through altered development of organisms. For instance, in mice, the sensation of sexual pheromones can induce endocrine changes in females that accelerate development and the onset of puberty (McLellan et al., 1998). We have investigated how sensory perception influences the growth and expression of alternative behavioral states of *C. elegans*.

Despite having a simple nervous system of 302 neurons, *C. elegans* is capable of perceiving and responding to a wide variety of environmental stimuli such as odorants, mechanical stimuli, food, osmotic, and ionic changes and pheromones. In *C. elegans*, environmental cues are detected through specialized sensory neurons. Sixty of the 302 neurons in *C. elegans* are ciliated sensory neurons, which are thought to be responsible for most sensory perceptions (Lewis and Hodgkin, 1977; Perkins et al., 1986). Like many sensory neurons in other animals, the sensory cilia of these neurons are specialized structures where environmental cues, including odorants and pheromones, interact with receptor proteins (Sengupta et al., 1996).

A class of mutants including *che-2*, *osm-6*, and *che-3* lack a normal sensory cilium structure (Lewis and Hodgkin, 1977; Perkins et al., 1986). The structural defects in these mutants are readily assessed because several ciliated sensory neurons take up vital dyes through the cilia, and these mutants fail to do so. As expected, mutants lacking cilia show diminished sensory responses to soluble and volatile chemicals. They have diminished responses to dauer pheromone, which induces a transition to an alternative nondeveloping dauer larva stage. It has also been reported that the cilium-defective mutants exhibit a longer life span than wild-type animals (Apfeld and Kenyon, 1999).

Here, we show that mutants with defects in cilium structure also exhibit abnormalities in the regulation of growth to a normal body size and in the expression of alternative states of locomotory behavior. The changes in body size in the cilium-defective mutants are not due to an inability to locate food. The results suggest that sensory perception can regulate neuroendocrine functions that determine the growth and ultimate body size of an organism. We also show that locomotory behavior of *C. elegans* in the presence of food is characterized by alternating behavioral states. In one state, the animal traverses widely separated regions of the plate (roaming), and in the other state, the animal restricts its activity to a confined region (dwelling). Analysis of cilium-defective mutants reveals that defects in sensory perception result in a relative decrease in the time spent roaming. Hence, the relative time spent roaming versus dwelling may be regulated by sensory perception.

We pursued a genetic analysis to determine how such changes in development and behavior are regulated by sensory perception. To identify neuronal mechanisms acting downstream of sensory perception in the regulation of these processes, we performed a screen for suppressor mutations of the *che-2* small body size pheno-

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type (*chb*). A subset of these suppressors also suppress the defect in locomotory behavior of *che-2*. Here, we show that one of these suppressors, *chb-1* (which is allelic to *egl-4*; Trent et al., 1983), encodes a cGMP-dependent kinase. A homologous cGMP-dependent protein kinase is expressed in vertebrate brain, although the physiological functions of this kinase in the nervous system have been controversial. Our results suggest that cGMP-dependent kinase is required for the processing of sensory information that is essential to multiple behavioral and developmental circuits in *C. elegans*.

Results

Mutants with Defects in Sensory Perception Have Smaller Bodies

Several mutants, including *che-2*, *che-3*, and *che-7*, are known to have morphological defects in sensory cilia. Lewis and Hodgkin (1977) first observed that some of these mutants have smaller bodies than wild-type animals. We have quantified the body size of these mutants from the L4 stage to late adulthood. Wild-type animals and three cilium-structure mutants, *che-2(e1033), che-3(e1124),* and *osm-6(p811)*, were raised at 20°C, and body size was measured at several time points over 120 hr. Body size was calculated by measuring the length of the perimeter of individual animals using digital images of worms. All of the cilium-structure mutants including *che-2(e1033)*, *che-3(e1124)*, and *osm-6(p811)* exhibit a smaller body size than wild-type animals at the L4 stage, which persists until growth ceases (Figures $1A_1$ and $1A_2$). The maximum body sizes of the cilium-structure mutants are approximately 83%–93% of the maximum body size of wild-type animals. To determine whether cell size was likely to be altered in cilium-structure mutants, we measured intranucleoli distances between seam cells (wild-type = 40.2 ± 0.9 μm; *che-2(e1033)* = 34.9 ± 1.6 μm). The results suggest that the change in body size in the cilium-structure mutants is at least partially due to smaller cell size. We also did not observe a change in the number of specific cell types based on an estimation of the number of cell nuclei with DAPI staining (data not shown).

Some of the cilium-structure genes encode proteins required for microtubule based transport in cilia (Qin et al., 2001). Although all of these genes appear to be expressed almost exclusively in sensory neurons, it is conceivable that other microtubule structures besides sensory cilia require these genes and are necessary for growth to a normal body size. We therefore also measured the body size of *tax-2(p671)* and *tax-4(p678)* mutants that have defects in sensory signal transduction because of mutations in genes encoding subunits of the cGMP gated channel (Coburn and Bargmann, 1996; Komatsu et al., 1996). These mutants also grow to a smaller body size (Figure $1A_2$), suggesting that sensory perception is required for growth to a normal body size. Another mutant, *kin-29*, also appears likely to be small due to defects in sensory perception (Lanjuin and Sengupta, 2002).

How might altered sensory perception lead to a change in maximum body size? Defects in sensory perception may have effects on the ability of an animal to locate food, resulting in a smaller size due to nutrient deprivation. This explanation is unlikely, however, because *che-2* mutants appear to be as healthy as wild-type animals, as indicated by a normal brood size. *che-2* animals also exhibit a normal pharyngeal pumping rate on food (Figure 1B). To further address this issue, we raised *che-2* mutants and wild-type animals in liquid cultures of bacteria from the L1 stage. Under these conditions, animals do not need to control their direction of movement to find food, ensuring the same food availability in wild-type animals and *che-2* animals. In liquid culture, both wild-type and *che-2* animals grow to a slightly smaller size than on plates, but *che-2* mutants are smaller than wild-type animals to a similar degree (76% of the body size of wild-type maximum) (Figure 1C). Together, these findings suggest that sensory perception may regulate body size independently of food localization.

Mutants with Defects in Sensory Perception Exhibit Altered Locomotory Behavior

Defects in cilium structure also result in altered locomotory behavior. We observed a distinctive change in the tracking patterns of the cilium-structure mutants in the presence of food. When a single wild-type animal is placed on a plate seeded with bacteria and allowed to move freely for 17–20 hr, the resulting tracking pattern spans the entire seeded region (Figure 2A). Under the same conditions, a *che-2*(*e1033)* mutant generates a track that is confined to a restricted area (Figure 2B). The other cilium-structure mutants *osm-6(p811)* and *che-3(e1124)* exhibit very similar tracks to those generated by *che-2* animals (data not shown). The sensory signal transduction mutants, *tax-4(p678)* and *tax-2(p671)*, show slightly less confined tracks (only *tax-4* shown, Figure 2C). This difference in tracking between *che-2* and N2 (wild-type) is not due to uncoordinated movement or inability to move. *che-2* animals are able to move smoothly at a normal rate when animals are mechanically stimulated by gentle touch (Figure 2D). However, without mechanical stimulation, the frequency of body bends during spontaneous forward movement is lower than in wild-type animals (Figure 2D). Interestingly, *egl-8(n488)* animals, which are known to have a low frequency of body bends and thus slower speed (similar in extent to *che-2*, Miller et al., 1999) (Figure 2D), generate tracks covering a much wider area than *che-2* (Figure 2E). This suggests that the tracking pattern of *che-2* cannot be explained solely by the reduced speed (frequency of body bends) of spontaneous movement. The defect resulting in the decreased speed of movement of *egl-8* is also likely to be distinct from that in *che-2*, as *egl-8* animals are unable to substantially increase their speed of locomotion after mechanical stimulation (Figure 2D).

In order to determine the cause of the confined tracks of *che-2* animals, we used a computerized image analysis system (DIAS) to analyze the movement of single animals. Movements of single animals on a plate seeded with bacteria were recorded up to 100 min with a digital video camera attached to a dissecting microscope. In Figure 3A, examples of wild-type and *che-2* tracking patterns are shown. This analysis of wild-type animals

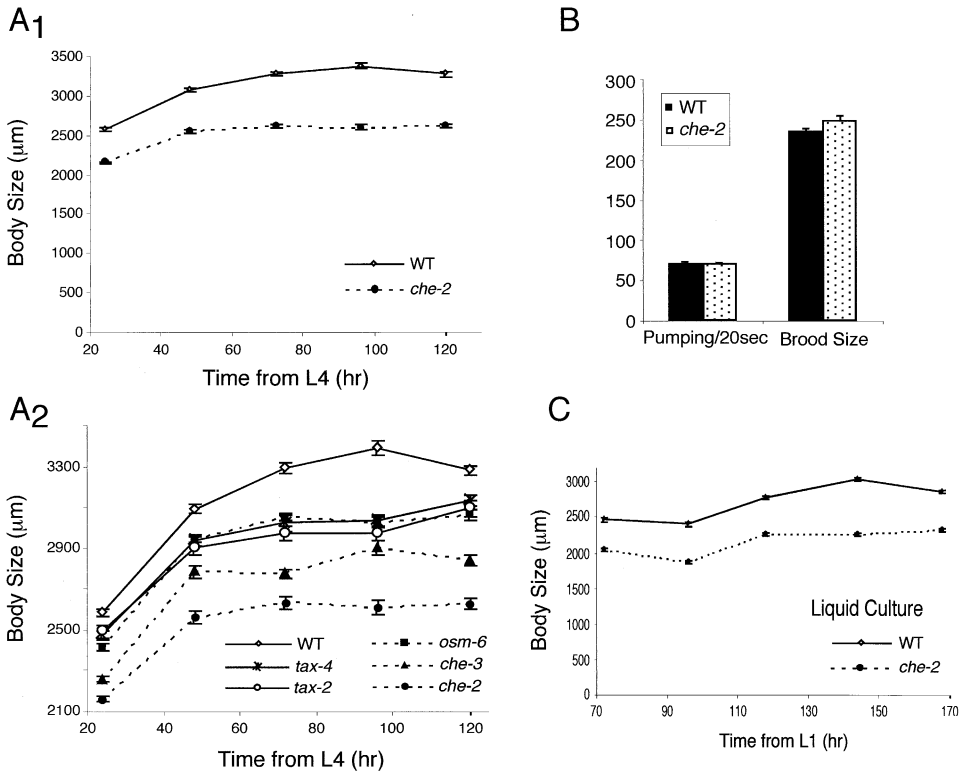

Figure 1. Sensory Cilium-Structure Mutants Exhibit Small Bodies

(A_1 and A_2) Developmental time course of body size at 20°C is shown. Body size (length of the perimeter of the body) was measured at indicated time points from the L4 stage. Each data point represents a mean of 20–30 animals, and error bars indicate the standard error of the mean (SEM). In (A_1), wild-type (N2) and *che-2(e1033)* are shown. In (A_2), wild-type (N2), *che-2(e1033)*, *che-3(e1124)*, *osm-6(p811)*, *tax-2(p671)*, and *tax-4(p678)* are shown (note that the scale of the *y* axis is changed from [A_1] to allow better comparison of the strains). Body size of all mutants (at 96 hr after the L4 stage) are significantly different from that of N2 ($P < 0.001$).

(B) Pharyngeal pumping was counted over 20 s intervals in adult animals. 30–34 counts were taken from at least 10 animals (24 hr after the L4 stage) for each strain. Total number of eggs laid by single animals were counted (number of animals, wild-type [N2] = 18, *che-2(e1033)* = 12). Means and SEM are shown. *che-2* is not significantly different from N2 in either pumping rate or brood size ($P > 0.05$).

(C) Body size in liquid culture. Time from newly-hatched L1 larva are shown. Each data point represents a mean of 20–30 animals, and error bars indicate the SEM. Body size of *che-2* (at 144 hr after the L1 stage) is significantly different from that of wild-type (N2) ($P < 0.001$).

revealed that locomotory behavior in the presence of bacteria consists of at least two behavioral states. As shown in Figure 3A, one state is characterized by a low speed with relatively more turning (observed at time (T): T0–57, T60–69, T77–82; average with SEM over this phase, 14.4 ± 0.5 μm/s, 63.8 ± 2.5°/5 s). The other state is characterized by movement at a high speed with relatively less turning (T57–60, T69–77, T82–90; 108.0 ± 3.6 μm/s, 12.8 ± 0.6°/5 s). We defined the low-speed/high-turning state during which the animal remains in a restricted area as dwelling and the high-speed/low-turning state during which the animal moves to distant areas as roaming. During dwelling, animals are observed to predominantly move forward, although backward movement and periods of no discernable movement are also frequently observed. The vast majority of the total area traversed occurs during a relatively small percentage of time when the animal is roaming (Figure 3A).

In *che-2*, as described above, the speed of movement is generally low compared to that of wild-type animals. Nevertheless, as shown in Figure 3A, a relatively high-speed/low-turning roaming state (T8–10, T78–79; 50.1 ± 2.9 μm/s, 10.0 ± 1.4 °/5 s) and a relatively low-speed/high-turning dwelling state (T0–8, T10–78, T79–100; 8.5 ± 0.2 μm/s, 69.7 ± 2.1°/5 s) are observed in *che-2*. The percentage of time spent in the roaming state appears quite short in *che-2* animals compared to wild-type animals. To quantify the differences, the locomotion of N2 animals was analyzed by a segmentation procedure (Pierce-Shimomura et al., 1999). Tracks were divided into segments called movements, defined as the periods between turns of at least 50°/5 s. A movement-duration histogram was computed (Figure 3B). The movement-duration histogram was best fit by the sum of two exponentials, which represent long and short movements, consistent with a model of two distinct behavioral states. The x value of the intersection of the two exponential lines gives the critical movement-duration value, t_{crit} (44 s), the value that best distinguishes between roaming and dwelling. We defined movements whose duration is greater or equal to 44 s as roaming and those shorter than 44 s as dwelling. Based on these criteria, the percentage of time spent roaming for each strain was determined. As shown in Figure 3C, time spent roaming is significantly decreased in *che-2* (7.5%) compared to N2 (21.4%). Taken together, our data indi-

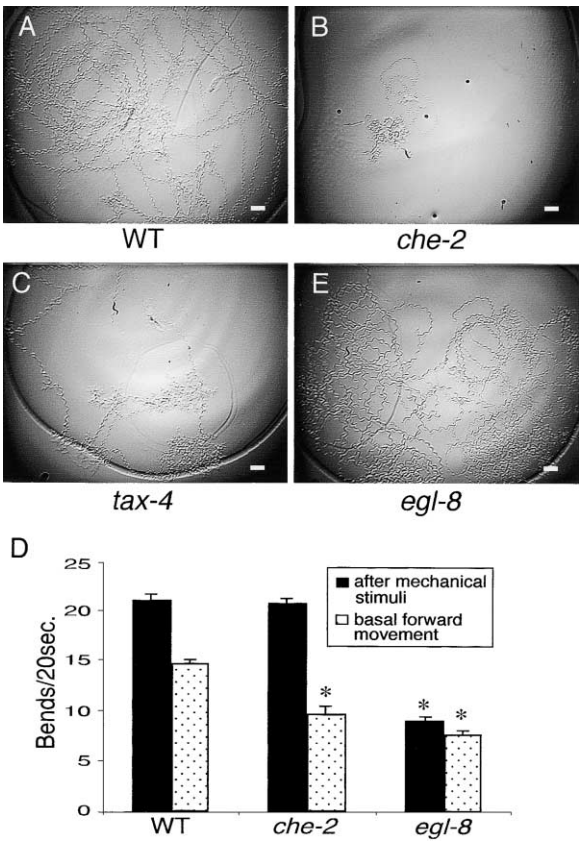

Figure 2. Sensory Cilium-Structure Mutants Exhibit Altered Locomotory Behavior

(A, B, C, and E) Tracks generated over 18 hr by single wild-type (N2), *che-2(e1033)*, *tax-4(p678)*, and *egl-8(n488)* animals. One example is shown for each strain. At least 50 plates for each strain were checked and showed similar patterns. Scale bar: 1 mm.

(D) Body bends/20 s of wild-type (N2), *che-2(e1033)*, and *egl-8(n488)* were counted with or without mechanical stimulation. Means (at least 20 counts using 10 animals for each strain) and SEM are shown. Asterisks mark responses that are different from N2 ($P < 0.001$).

cate that the difference in tracking between *che-2* and wild-type animals is due to a relative decrease in the time roaming as well as slowing of movement. This suggests that locomotory behavior on food is characterized by alternating behavioral states (roaming/dwelling) that may be regulated by environmental cues perceived through the sensory-ciliated neurons in wild-type animals.

A number of factors are known to modulate locomotory behavior of *C. elegans*. Wild-type animals move faster in the absence of food than in the presence of food (de Bono and Bargmann, 1998). We observe that *che-2(e1033)* also exhibits accelerated speed in the absence of food (data not shown), suggesting that *C. elegans* can detect food through *che-2*-independent sensory functions. It is unclear whether states analogous to roaming and dwelling exist in the absence of food. Another recent study has analyzed the effect of egg-laying events on locomotory behavior on food. The speed (velocity) and reversal (defined as turns >120°) frequency were altered by egg-laying events (Hardaker et al., 2001). Analysis of velocity changes showed non-random fluctuations that could be consistent with multiple states, although states analogous to roaming and dwelling were not identified (Hardaker et al., 2001).

Regulation of Body Size through Sensory Perception by Amphid Neurons

C. elegans has 60 ciliated sensory neurons and *che-2* mutants are observed to lack the cilia of all ciliated sensory neurons except for the AFD neurons (Lewis and Hodgkin, 1977). We wanted to understand which sensory neurons are important for the regulation of body size and locomotory behavior. We expressed *che-2* cDNA in specific neurons using a variety of different promoters. In this way, function could be restored only in a particular sensory neuron or subset of neurons because *che-2* acts cell autonomously (Fujiwara et al., 1999).

As a control, the *che-2* gene was expressed in all ciliated sensory neurons in a *che-2* mutant, using a *che-2* promoter (Table 1). A significant increase in body size and restoration of locomotory behavior was confirmed (see also Table 1 legend). *che-2* was then expressed in amphid neurons. The amphid, which is comprised of 24 ciliated sensory neurons, is the principal sensory organ of *C. elegans*. We used the *tax-4*, *gpa-3*, and *odr-3* promoters for amphid expression of *che-2* (Table 2; Komatsu et al., 1996; Zwaal et al., 1997; Roayaie et al., 1998). Expression of *che-2* by any of these promoters rescued the body size to a similar extent as the *che-2* promoter (Table 1). Among these promoters, the *odr-3* promoter gives expression in the smallest subset (five pairs) of amphid neurons, suggesting that sensory perception by these amphid neurons is sufficient for body size regulation. In addition, functional redundancy was suggested, because the *tax-4*, *gpa-3*, and *odr-3* promoters generate expression in overlapping sets of amphid neurons, but there are no neurons in which all three promoters drive expression. More specific promoter constructs that give *che-2* expression in AWB and AWC (*odr-1* promoter), AWA (*odr-10*), ASH and ASI (*sra-6*), ASJ (R09F10.6), and ASK (*sra-9*) (Yu et al., 1997; Sengupta et al., 1996; Troemel et al., 1995) failed to rescue the body size defect (data not shown).

In terms of locomotory behavior, the *odr-3* promoter construct does not rescue the defect in locomotory behavior siginificantly. The *tax-4* or *gpa-3* promoter constructs rescue the defect but only partially (Table 1). Introduction of both *tax-4* and *gpa-3* promoter constructs improves rescue of the locomotory behavior defect (Table 1). These results suggest that the normal locomotory behavior may require amphid neurons, but it may be a larger subset than that required for body size regulation.

Genes Acting Downstream of the *che-2*-Dependent Sensory Input

To identify the genes required for the processing of sensory inputs that regulate body size, we performed a screen for *che-2* small body size suppressors (*chb*). *che-2(e1033)* animals were mutagenized with EMS, and F2 progeny exhibiting an increased body size were isolated. Screening of approximately 15,000 haploid genomes

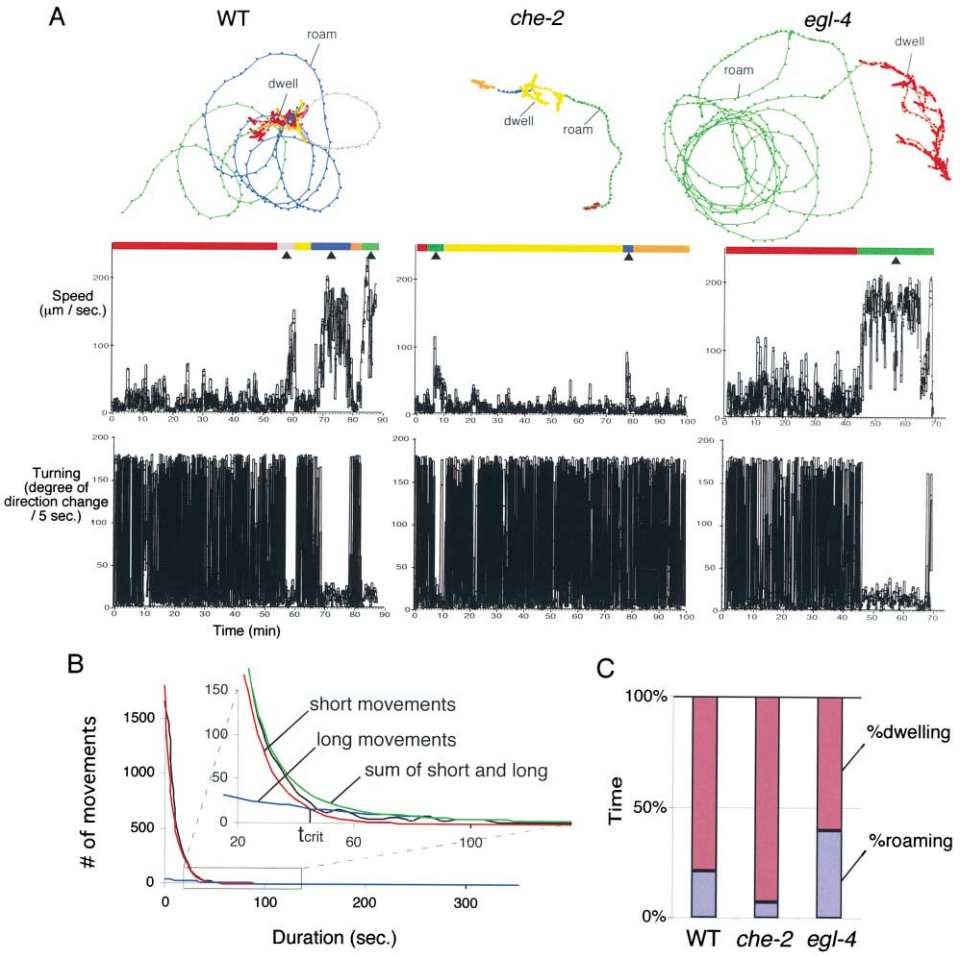

Figure 3. Speed and Direction Change Analysis

Movements on bacterial lawn of wild-type (N2), *che-2(e1033)*, and *egl-4(eg64)* were recorded for up to 100 min with a digital video camera and then analyzed with DIAS.

(A) One example of analysis for each strain. Upper: the animal positions (centroids) are presented as a dot every 5 s. Colors of tracks correspond to the colors of time bars in speed graphs. At least one dwell state and one roam state is indicated in each plot. Middle: speeds at 5 s intervals are plotted over time. Triangles under time bar indicate time periods when an animal is roaming. Bottom: direction changes over 5 s are plotted over time.

(B) Histogram of movement durations (bin = 5 s) of wild-type (N2). Movements were defined as track segments between sharp turns, which are defined as a direction change greater than 50°/5 s. Blue and red exponential lines represent the predicted long- and short-movement distributions, respectively. The movement-duration histogram (shown as black line) is fit by the sum (shown as green line) of the two exponentials. The x value of the intersection of the two exponential lines indicates the critical movement duration (t_{crit} = 44 s) (minutes and the number of animals observed; N2, 1278 min [n = 12]).

(C) Percentage of time roaming and dwelling. By using t_{crit} = 44 s from (B), % time of roaming (long movements) and dwelling (one or more consecutive short movements) was calculated for each strain based on their direction change data. Percentage of time roaming of *che-2* and *egl-4* are different from that of wild-type (N2) at $P < 0.001$ by chi-square analysis. (Minutes and the number of animals observed; N2, 1278 min [n = 12]; *che-2(e1033)*, 562 min [n = 6]; *egl-4(eg64)*, 521min [n = 6]).

yielded 23 *chb* suppressor mutants. These mutants in a *che-2* background still showed defective dye-filling, indicating that all of these suppressor mutations suppress the small body size of *che-2*, but not the cilium structural defects of *che-2*. One of these suppressors, *chb-1* (which was found to be allelic to *egl-4*, see below), resulted in a larger body size than wild-type (Figure 4A).

Our primary interest is in genes acting downstream of sensory inputs. *chb* suppressors could, however, act in a pathway regulating body size that is parallel to the pathway in which *che-2* functions. The *che-2*;*chb* double mutant may then have a normal body size as a result of additive effects. In this case, the *chb* single mutant is likely to be larger than the double mutant. Such mutants are defined as class II. If, on the other hand, a *chb* suppressor acts downstream of the *che-2*-dependent sensory input, the combined effect of *che-2* and the suppressor mutations is most likely to result in a body size equivalent to that resulting from the suppressor mutation alone. Such mutants are defined as class I. We analyzed the 23 suppressor lines based on these criteria (see Experimental Procedures). We found both class I (eight alleles) and class II (15 alleles) suppressors. By further analysis, we identified four loci in class I:

Table 1. Sensory Neurons Required for Normal Body Size and Locomotory Behavior

Promoter for *che-2* Expression	*che-2*	*tax-4*	*gpa-3*	*tax-4/ gpa-3*	*odr-3*	*egl-4.a*
Body size	+	+	+	+	+	+
(μm; nontransformant,	2428 ± 16	2401 ± 16	2427 ± 19	2505 ± 34	2490 ± 15	2514 ± 28
transformant)[A]	2662 ± 22	2581 ± 28	2641 ± 19	2636 ± 24	2700 ± 32	2736 ± 19
Track[B]	+	+/−	+/−	+	−	+
(grid squares entered;	4.9 ± 0.7	5.6 ± 0.6	5.6 ± 0.6	3.9 ± 0.4	6.0 ± 0.5	3.4 ± 0.6
nontransformant, transformant)	17.2 ± 1.0	9.6 ± 1.0	8.3 ± 0.8	15.4 ± 2.8	7.2 ± 0.6	13.6 ± 1.2

[A] Average of the body size at 48 hr after the L4 stage with SEM. "+" indicates rescue (different from control [nontransformant measured at the same time] at $P < 0.01$). The body size of wild type (N2) at the same stage is 2984 ± 20, suggesting that even expression by the *che-2* promoter dose not rescue completely.

[B] Tracking assays were done as described in the Experimental Procedures. Tracks generated on food over a 17–20 hr period were superimposed on a 5 mm grid (5 × 5), and the number of squares an animal entered was counted. Average with SEM of test strains (transformant) and control (nontransformant, assay was done simultaneously with test strains) are shown. The average score of wild type (N2) is 21.6 ± 1.2, again suggesting that rescue by *che-2* promoter is not complete. Both "+" and "+/−" indicate a significant difference from control at $P < 0.01$. Rescue is more complete for "+" (three to four times larger than control) than for "+/−" (less than two times control).

egl-4 (LG IV left; *eg36, eg43, eg44, eg64*), *chb-2* (LG II middle; two alleles), *chb-3* (LG I left; one allele), and *chb-4* (LG V right; one allele) (data only shown for *egl-4*, Figure 4A).

Class I Genes Also Act in the Regulation of Locomotory Behavior

Interestingly, all class I genes were found to suppress not only the defect in body size, but also the altered locomotory behavior of *che-2* (data only shown for *egl-4*, Figure 4B). None of the class II genes suppresses the locomotory defect of *che-2* (data not shown). We analyzed the locomotory behavior of the *egl-4* single mutant using DIAS. *egl-4(eg64)* was found to have the opposite phenotype of *che-2*; these animals exhibit a significant increase in the percent of time spent roaming (N2, 21.4%; *egl-4*, 39.5%) and accelerated speed (roaming 144.2 ± 2.8 μm/s; dwelling 28.0 ± 1.1 μm/s) (Figures 3A and 3C). It is difficult to determine whether the higher speed of *egl-4* is due to the larger body size of *egl-4* or an actual change in behavior. The increase in roaming time must, however, reflect altered behavior. The increase in roaming time is not equivalent to a hyperactive phenotype, which has been defined as a higher frequency of body bends. We tested one hyperactive mutant, *egl-30(js126gf)* (Brundage et al., 1996), which has an increase in frequency of body bends but proved to have a slight decrease rather than an increase in the roaming time (data not shown). These results suggest that the class I genes are involved in the regulation of locomotory behavior as well as body size.

egl-4 Encodes a cGMP-Dependent Protein Kinase

egl-4 alleles were isolated as one of the class I suppressors, originally named as *chb-1*. These *chb-1* alleles were mapped to the same chromosomal region as *egl-4* mutants. *chb-1* failed to complement *egl-4* (based on chemotaxis behavior, data not shown). *chb-1* animals share all of the known phenotypes of *egl-4* animals including a larger body size, inappropriate dauer formation at high temperature, decreased sensitivity to aldicarb (an acetylcholinesterase inhibitor), and defects in egg-laying behavior and chemotaxis (see below; Trent et al., 1983; Daniels et al., 2000). These phenotypes are known to be caused by defects in the nervous system (Sze et al., 2000; Ailion and Thomas, 2000) and are consistent with our expectation that *egl-4* functions in the nervous system, probably downstream of sensory inputs, as a class I suppressor.

We mapped *egl-4* using the snip-SNP method (Wicks et al., 2001) based on its suppressor phenotype of the altered locomotory behavior of *che-2* (Figure 4C). The mutation was mapped to an interval of approximately 90 kb on LG IV left (Figure 4C). According to the *C. elegans* genome sequence, this region includes four predicted genes. A combination of cosmids (W03A6 and K07A9), which covers one of the genes (F55A8.2), showed rescuing activity for the chemotaxis defect to diacetyl. A full-length cDNA for F55A8.2 also showed rescuing activity for all phenotypes of *egl-4* (see below). Six full-length cDNA clones for F55A8.2 (325g6, 328c10, 341c9, 479h11, 87g12, and 85a3, obtained by Yuji Kohara cDNA project) were examined. 325g6, 328c10, 341c9, and 479h11 had identical digestion patterns (data not shown). Sequencing of 325g6 showed a gene structure consisting of ten exons, 2343 bp in size (780 amino acids). This transcript was named *egl-4.a* (Figure 5). One cDNA clone, 87g12, has a different first exon starting from about 1.6 kb downstream of the first exon of *egl-4.a*, but shares the second to tenth exons with *egl-4.a*.

Table 2. Cellular Patterns of Expression of *tax-4, gpa-3,* and *odr-3*

Promoter	Cellular Patterns of Expression
tax-4	AWB, AWC, AFD, ASE, ASG, ASI, ASJ, ASK, BAG, URX
gpa-3	ASE, ADF, ASG, ASH, ASI, ASJ, ASK, ADL, PHA, PHB, PVT, AIZ
odr-3	AWB, AWC, AWA*, ADF*, ASH*

* Indicates weak expression.

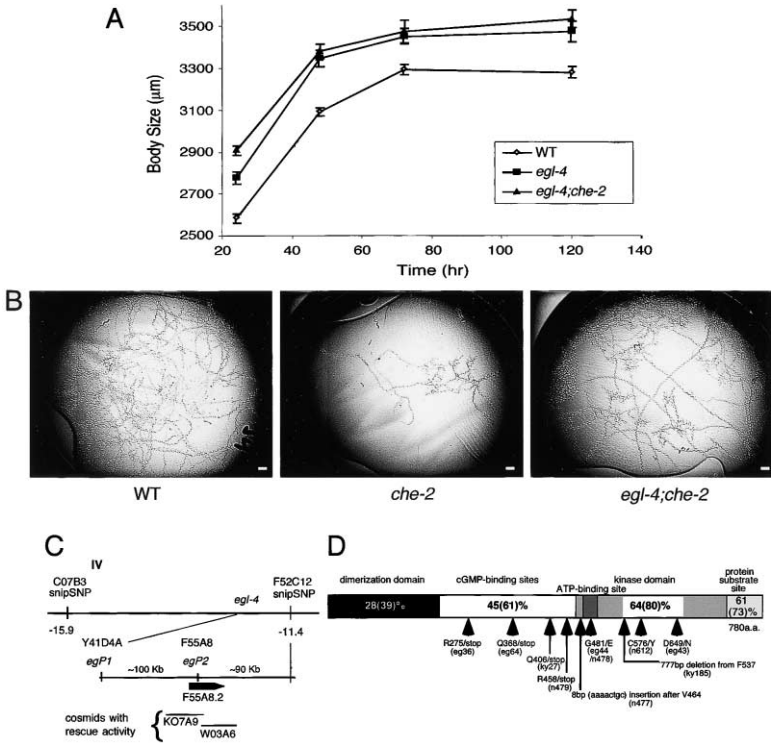

Figure 4. *egl-4*, a Class I *che-2* Small Body Size Suppressor

(A) *egl-4;che-2* double mutants are larger than wild-type and the same size as *egl-4* single mutants. Body sizes of wild-type (N2), *egl-4(eg64)* single mutants, and *egl-4 (eg64);che-2(e1033)* double mutants were measured as described in Figure 1A. Body sizes of *egl-4(eg64)* and *egl-4(eg64);che-2(e1033)* are significantly different from that of N2 at $P < 0.001$ (at 120 hr after the L4 stage). Body size of *egl-4(eg64)* is not different from that of *egl-4(eg64);che-2*(e1033) ($P > 0.05$, at 120 hr after the L4 stage) (note that the *y* axis starts from 2500 μm to allow better comparison of the strains).

(B) *egl-4* also suppresses the altered locomotory behavior of *che-2*. Wild-type (N2), *che-2(e1033)*, and the *egl-4(eg64);che-2(e1033)* double mutant were assayed for tracking as described in Figure 2. Scale bars: 1 mm.

(C) Genetic and physical maps of the *egl-4* region. See text. (note that snip-SNPs were identified by S. Wicks [Wicks et al., 2001], except egP1 and egP2, which were identified by M.F.).

(D) *egl-4* encodes a cGMP-dependent protein kinase. The *egl-4* protein sequence based on the *egl-4.a* cDNA clone (see below) was compared with the human cGMP-dependent protein kinase type-Iβ (PID;g10835242) sequence. Percentage of identical amino acids (and similar amino acids in parentheses) is shown for each domain. Mutation sites of all alleles of *egl-4* are also shown.

This transcript was named *egl-4.b* (2214 bp, 737 amino acids) (Figure 5). The last cDNA clone, 85a3, has a gene structure which is the same as *egl-4.a*, except for a missing eighth exon. BLAST search analysis suggests that the *egl-4* gene encodes a highly conserved cGMP-dependent protein kinase homolog (Figure 4D).

Ten alleles were sequenced for mutation sites in F55A8.2 (Figure 4D). Six alleles are possible null alleles whose products lack the kinase domain as a result of early nonsense mutations (*eg36, eg64, ky27,* and *n479*) or deletion (*ky185*) or an insertion that would cause a frame shift (*n477*). *n478*, the reference allele, has a missense mutation in the ATP binding site of the kinase and exhibits the most severe chemotaxis defect (Daniels et al., 2000), supporting the importance of the kinase activity for function.

egl-4 Acts in Sensory Neurons to Regulate Body Size and Locomotory Behavior

In our model, the EGL-4 kinase acts downstream of *che-2*-dependent sensory inputs to regulate body size and locomotory behavior. To confirm that EGL-4 kinase functions in the nervous system, the *egl-4.a* cDNA (the major cDNA form) was fused to a pan-neuronal promoter (H20). This fusion gene was introduced into *egl-4(eg64)* and *egl-4(eg64);che-2(e1033)*, and resulting transgenic animals were analyzed (Figure 6). *egl-4* mutants bearing the H20::*egl-4.a* transgene were rescued for chemotaxis, egg laying, and dauer formation. *egl-4;che-2* mutants bearing the H20::*egl-4.a* transgene exhibit the *che-2*-like small body and confined tracks, indicating the suppressor phenotypes in body size and locomotory behavior were rescued. We also tested the *tax-4, gpa-3*,

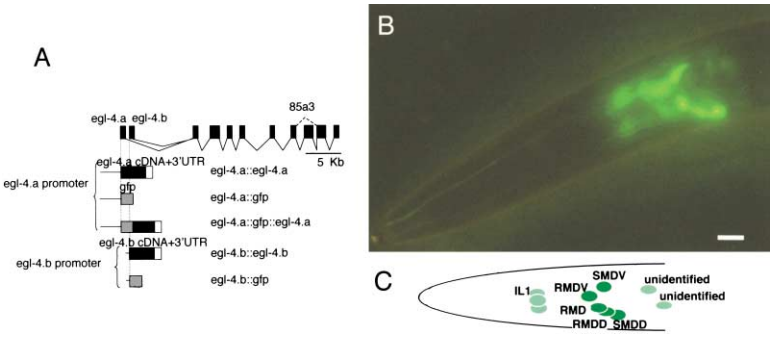

Figure 5. *egl-4* Is Expressed in Neurons

(A) The cDNA expression constructs and GFP reporter constructs are shown under the gene structure.

(B and C) Expression pattern of the *egl-4.a*::gfp construct. The head region is shown (anterior is to left). Although we observed expression in several other neurons (six in the ventral nerve cord), only neural expression was observed. Scale bar: 10 μm.

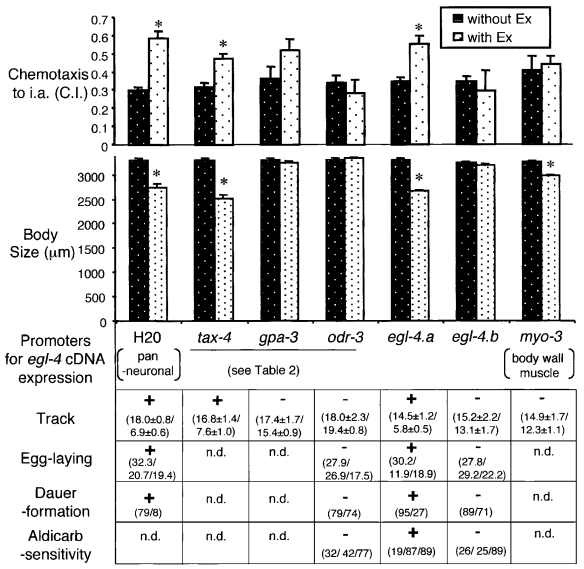

Figure 6. *egl-4* Acts in Neurons to Regulate Body Size and Locomotory Behavior

The *egl-4* cDNA under the control of various promoters was tested for rescuing activity of *egl-4* mutant phenotypes. Each transgene was introduced into mutants as extrachromosomal arrays with GFP coinjection markers. We compared control animals (without Ex) and animals with the transgene (with Ex) using fluorescence. Asterisks and pluses indicate rescue (different from control, $P < 0.001$).

From top: chemotaxis to isoamyl alcohol; each transgene was introduced into *egl-4 (eg64)*. Assays were performed using a mixed population of control animals and animals with the transgene. The animals were then scored separately under a fluorescence microscope to determine chemotaxis indexes. Means and SEM of at least six assays for each line are shown (note that the expression of *egl-4* by the the *odr-3* promoter, which gives expression in AWC, failed to rescue the chemotaxis defect to isoamyl alcohol, an AWC-sensed odorant. The chemotaxis defect to another odorant, butanone, which is also sensed by AWC, was rescued by the same construct (data not shown, also see L'Etoile et al., 2002), suggesting that the promoter construct was expressed in AWC but that there is a more complicated mechanism for isoamyl alcohol chemotaxis).

Body size: each transgene was introduced into *egl-4 (eg64);che-2(e1033)*. Average body size (at 48 hr after the L4 stage) of control animals and animals with the transgene are shown. Error bar indicates SEM.

Track: each transgene was introduced into *egl-4(eg64);che-2(e1033)*. Tracks left by control animals and animals with the transgene were scored. See Table 1 legend for the scoring method (parentheses; average score with SEM of animals without transgene/those with transgene).

Egg laying: each transgene was introduced into *egl-4(eg64)*. The number of eggs in a uterus was compared for control animals and animals with the transgene (parentheses; means of 20–40 animals without transgene/those with transgene/N2).

Dauer formation at 27°C: each transgene was introduced into *egl-4(eg64)*. A mixed population of control animals and animals with the transgene were cultured at 27°C, and ~100 nondauers and ~100 dauers were isolated for scoring % of animals with transgene (parentheses; % of animals with transgene in 100 nondauers/in 100 dauers).

Aldicarb sensitivity: each transgene was introduced into *egl-4(eg64)*. Aldicarb-induced paralysis was compared for control animals and animals with the transgene (parentheses; % of paralyzed animals without transgene/those with transgene/N2).

See Supplemental Data for additional details of behavioral assays.

and *odr-3* promoters that generate expression of the *egl-4.a* cDNA more selectively in sensory neurons (Table 2). Among these, the *tax-4* promoter construct showed rescuing activity in terms of the defects in chemotaxis, body size, and locomotory behavior (Figure 6). These results indicate that EGL-4 kinase acts in the nervous system, particularly in sensory neurons.

We also assessed the expression pattern of several *egl-4* GFP reporter fusion constructs. The 2.8 kb upstream promoter region of *egl-4.a* (*egl-4.a* promoter) was fused to gfp cDNA (*egl-4.a::gfp,* Figure 5A). This *egl-4.a* promoter rescued all tested phenotypes when fused to the *egl-4.a* cDNA (*egl-4.a::egl-4.a*) (Figure 6). The *egl-4.a::gfp* construct is expressed in several head neurons (IL1 sensory neurons, SMD, and RMD motor/interneurons, and two unidentified neurons), and occasional weak expression is observed in six ventral cord motor neurons (possibly VC neurons) from the late embryonic stage through the adult stage (Figures 5B and 5C). We also made another reporter construct by inserting gfp cDNA into the *egl-4.a*::*egl-4.a* construct in frame (*egl-4.a::gfp::egl.4-a*; Figure 5A). This construct gives essentially the same expression as *egl-4.a::gfp*, but occasionally we observed additional faint expression in several unidentified neurons including amphid sensory neurons. Although the GFP expression in sensory neurons is variable and faint (except in IL1), we obtained additional evidence, indicating that the *egl-4.a* promoter generates expression in sensory neurons; when *che-2* cDNA is fused to the *egl-4.a* promoter (*egl-4.a*::*che-2*) and introduced into *che-2* mutants, the transgenic animals showed uptake of dye in amphid neurons (data not shown). Rescue of the abnormalities in body size and locomotory behavior was also observed (Table 1).

The 1.4 kb upstream region of *egl-4.b* (*egl-4.b* promoter, Figure 5A) was fused to gfp cDNA (*egl-4.b::gfp*) and expression in body wall muscle was observed (data not shown). The *egl-4.b* cDNA under the control of the *egl-4.b* promoter (*egl-4.b::egl-4.b*) does not show any rescuing activity (Figure 6). However, we cannot exclude a possible minor role of this kinase in muscle for body size regulation because *egl-4.a* cDNA expression in muscle by the *myo-3* promoter (provided by A. Fire) results in partial rescue of the body size defect (Figure 6).

Taken together, the results suggest that function of EGL-4 kinase in the nervous system, particularly in sensory neurons, is important for the regulation of body size and locomotory states as well as certain other phenotypes such as chemotaxis.

EGL-4 May Regulate Body Size through the DBL-1 TGF-β Cascade

Previous studies have shown that the DBL-1 TGF-β cascade plays an important role in body size regulation of *C. elegans*. The mutations in genes encoding the signaling components of this cascade, including *dbl-1* (TGF-β ligand) and *sma-6* (TGF-β type I receptor), cause a small body size (approximately 50% of wild-type) (Patterson and Padgett, 2000), which is more severe than that caused by *che-2* mutations. DBL-1 is expressed in neurons and appears to activate a SMA-6 receptor, which is known to act in the hypodermis to control the expression of genes regulating body size (Suzuki et al.,

1999; Yoshida et al., 2001). Recently, *lon-1* was identified as one of the genes which is negatively regulated by DBL-1. *dbl-1* loss-of-function mutants exhibit an increase in *lon-1* transcript levels and animals overexpressing *dbl-1* exhibit a decrease in *lon-1* transcript levels (Morita et al., 2002). Mutations in *lon-1* cause a thin and long body size (Morita et al., 2002).

Our genetic interaction experiments suggests that EGL-4 may control body size by regulating DBL-1. First, the large body size of *egl-4(ky185)* is fully suppressed by *dbl-1(wk70)*. The body size of the double mutant is similar to or slightly smaller than the *dbl-1* single mutant (average body size with SEM at 48 hr after L4 stage: *egl-4(ky185)* = 3262 ± 22 μm, *dbl-1(wk70)* = 1751 ± 14 μm, *egl-4(ky185);dbl-1(wk70)* = 1678 ± 11 μm). Second, *egl-4(eg64)* and *lon-1(e185)* show no synergy in combination. The *lon-1(e185);egl-4(eg64)* double mutant has the same body size as the *lon-1(e185)* single mutant (*lon-1(e185)* = 3958 ± 26 μm, *egl-4(eg64)* = 3209 ± 28 μm, *lon-1(e185);egl-4(eg64)* = 3936 ± 24 μm). We also measured the body size of *che-2(e1033);lon-1(e185)*. The double mutant exhibited an intermediate body size (*lon-1(e185)* = 4129 ± 27 μm, *che-2(e1033)* = 2498 ± 22 μm, *lon-1(e185);che-2(e1033)* = 3323 ± 56 μm), suggesting that the body size defect of *che-2* was not fully suppressed by the *lon-1* mutation. This result suggests that either *che-2* may act in parallel to the DBL-1 pathway or *lon-1* may not be the exclusive output of the DBL-1 cascade. Genetic data support the latter possibility because *lon-1(e185);dbl-1(nk3)* double mutants also exhibit an intermediate body size (Morita et al., 2002) similar to the *lon-1;che-2* double mutant. All of these results suggest that the EGL-4 kinase may act downstream of *che-2*-dependent sensory inputs to suppress DBL-1 TGF-β function in the nervous system.

Discussion

Sensory perception allows an organism to adapt to particular environmental conditions through changes in behavior and development. We have demonstrated that sensory neurons of *C. elegans* may regulate both the growth of the animal and the expression of alternative behavioral states. Through a genetic suppressor screen, we identified genes likely to be necessary downstream of sensory inputs for the information processing required for body size regulation. All of these class I genes also regulated the expression of particular alternative behavioral states, suggesting that the same neural or neuroendocrine mechanism regulates both growth and behavioral state. Here, we have characterized one of these genes, *egl-4*, and demonstrated that it encodes a cGMP-dependent protein kinase. We show that this kinase acts in sensory neurons of *C. elegans*. We propose that this cGMP-dependent protein kinase regulates the processing of sensory inputs that is required for multiple behavioral and developmental functions in *C. elegans*.

Sensory Perception Regulates Body Size

Determination of body size is one of the fundamental aspects of the development of an organism, although the underlying mechanisms are largely unknown (Conlon and Raff, 1999). Recent studies indicate that the growth of individual cells is not passively determined by the availability of nutrients but is controlled by monitoring the extracellular environment. TOR kinase, which is conserved from yeast to mammals, is known to act as a nutrient sensor and regulate protein synthesis to control cell mass (Schmelzle and Hall, 2000).

In higher systems, growth of individual cells and body parts must also be carefully orchestrated. In mammals, body growth is known to be regulated by neuroendocrine factors such as growth hormone and the insulin cascade. Recent studies have, in fact, suggested that insulin enhances the activity of mTOR via protein kinase B (Schmelzle and Hall, 2000). In *C. elegans*, body size changes are thought to be primarily dependent on cell size rather than cell number due to an essentially invariant cell lineage. As in higher systems, cell size and body size are also likely to be under neural or neuroendocrine control in *C. elegans*. Defects in a DBL-1 TGF-β cascade are known to cause dwarf phenotypes in *C. elegans* (Patterson and Padgett, 2000). This DBL-1 TGF-β is expressed mainly in motor neurons in *C. elegans* (Suzuki et al., 1999; Morita et al., 1999).

Our results suggest that the nervous system may not only orchestrate the growth of diverse cell types, but also provide a mechanism to adjust body size to environmental factors such as nutrition, temperature, or pheromones. We have shown that diminished sensory perception as a result of sensory cilium-structure defects results in a small body size in *C. elegans*. We show that the small body size does not reflect an inability to locate food due to the defects in sensory perception. Sensory perception may alter neural or neuroendocrine functions that determine body size. We can conclude that body size is regulated by sensory perception, independently of food localization.

C. elegans Exhibits Alternative States of Locomotory Behavior Regulated by Sensory Perception

By analyzing the temporal pattern of movement on food by computerized image analysis, we observed alternative states of locomotory behavior of *C. elegans*. Distinct behavioral states in *C. elegans* have also been described for egg-laying behavior (Waggoner et al., 1998). Most other behavioral genetic studies in *C. elegans*, however, have focused primarily on behavioral responses to experimental stimuli such as the response to mechanical stimulation or to an applied odorant. In higher systems, many aspects of behavior also involve transitions between alternative states such as mood, aggression, or appetitive behavior that are difficult to define as purely responses to discrete external stimuli. Such behaviors appear to also reflect additional internal or motivational factors. Identifying genes that regulate the expression of such alternative behavioral states has proven challenging in both vertebrate and invertebrate systems. The temporal pattern of locomotory behavior of *C. elegans* involves alternative states of roaming and dwelling, which appear to be generated without discrete stimuli on a uniform bacterial lawn but modulated by sensory perception through a subset of ciliated neurons. One possible interpretation is that without sensory inputs

through cilia, *che-2* animals have a decreased motivation to roam.

EGL-4, a cGMP-Dependent Protein Kinase, Acts in the Nervous System to Regulate Body Size and Behavioral State

By genetic screening for *che-2* small body size suppressors (*chb*), we identified mutations in four loci that are likely to have defects in the information processing required downstream of sensory inputs for body size regulation (class I). These mutants act as if they receive sensory inputs even when cilium structure remains defective due to background *che-2* mutations. Curiously, all of these class I body size suppressors (and none of the class II suppressors) also suppress the locomotory defect in *che-2* mutants. Therefore, a common neural circuit or signaling cascade is likely to regulate both body size and behavioral state. The growth to a smaller body size and decreased roaming could both represent adaptations to a perceived depletion of environmental resources; such changes in behavior and growth may represent a means to decrease energy utilization. Molecular cloning has revealed that one of the *chb* suppressors encodes EGL-4, a cGMP-dependent protein kinase (also see L'Etoile et al., 2002 [this issue of *Neuron*]). Recent biochemical analysis of this protein has indicated that this protein indeed has cGMP-dependent protein kinase activity (Stansberry et al., 2001). Our mutation site analysis also suggests the importance of the kinase activity.

In mammals, cGMP-dependent protein kinases are expressed in many different tissues including smooth muscle, platelets, intestine, and brain (Pfeifer et al., 1999). The function of these kinases in the nervous system has been controversial, and few neural substrates have been documented. Biochemical and cellular analysis has implicated cGMP-dependent protein kinases in long-term depression (LTD) in the cerebellar cortex and long-term potentiation (LTP) in the hippocampus (Lev-Ram et al., 1997; Zhuo et al., 1994). In contrast to these experiments, cerebeller LTD and hippocampal LTP are not attenuated in mice deficient in cGMP-dependent protein kinase (Kleppisch et al., 1999). However, more recent studies indicate another role of this kinase in synaptic plasticity, which is short-term facilitation in nerve terminals through BK channel modulation (Klyachko et al., 2001).

In *Drosophila*, strains having less cGMP-dependent protein kinase activity have been reported to move less during foraging (Osborne et al., 1997). Although the same kinase influences the movement of both species, the mechanisms may be different. In *Drosophila,* the general locomotory activity level during foraging is different in distinct strains. In *C. elegans*, transitions between alternative active and inactive states are affected in a single strain. The effects in *Drosophila* are also opposite in character; a decrease in this protein results in increased roaming in *C. elegans* but less movement in *Drosophila*. The expression of a cGMP-dependent protein kinase in *Drosophila* is observed in neuronal and nonneuronal tissues (Sokolowski, 1998). It is not yet known where expression is required for the regulation of foraging behavior of *Drosophila* (Osborne et al., 2001).

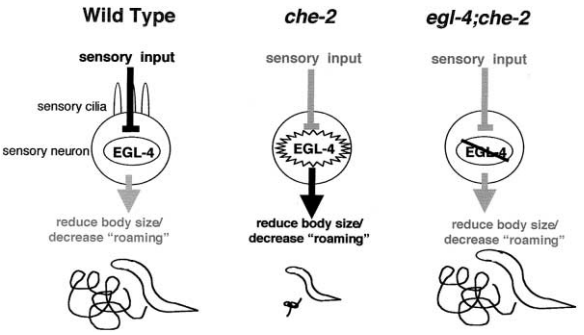

Figure 7. Model for the Role of EGL-4, a cGMP-Dependent Protein Kinase, in the Regulation of Body Size and Locomotory States (See Text for Details)

Although in this figure EGL-4 is shown to act in the same sensory neurons that receive the environmental cues for body size and locomotory regulation, EGL-4 could also act in different sensory neurons that form a neural circuit with the sensory neurons receiving the environmental cues).

Our data suggest that EGL-4, a cGMP-dependent protein kinase, acts in the nervous system and particularly in sensory neurons in *C. elegans*. This represents a novel demonstration of the role of a cGMP-dependent protein kinase in sensory neurons for modulation of sensory information (also see L'Etoile et al., 2002). A previous study reported that *egl-4* mutant phenotypes including enhanced dauer formation at high temperature, large body size, egg-laying defects, and even chemotaxis defects were suppressed by *daf-3* mutations, suggesting that *daf-3* functions downstream of *egl-4* (Daniels et al., 2000). *daf-3* encodes a member of the SMAD protein family acting downstream of the *daf-7* TGF-β cascade and is expressed in many neurons, the intestine, the pharynx, and hypodermis (Patterson et al., 1997). We observed additional genetic interactions with *dbl-1* and *lon-1*, suggesting that *egl-4* may act upstream of the *dbl-1* TGF-β cascade, which is a separate cascade from the *daf-7* TGF-β cascade. Although it is unclear whether *egl-4* directly regulates *dbl-1* and how *daf-3* may interact with the *dbl-1* TGF-β cascade, the results suggest that the EGL-4 cGMP-dependent kinase may link sensory perception to the *dbl-1*TGF-β cascade.

Here, we propose a model (Figure 7) in which sensory perception, acting through modulation of a cGMP-dependent kinase, regulates the growth and locomotory behavior of the animal. Normally, the EGL-4 cGMP-dependent kinase functions to reduce body size and decrease roaming. In wild-type animals, EGL-4 activity would be inhibited by sensory inputs. In *che-2* mutants, EGL-4 would be inappropriately activated, resulting in a small body and decreased roaming. In *egl-4*;*che-2* double mutants, EGL-4 function is eliminated, and body size and roaming are increased. Such a model of inhibitory sensory inputs is reminiscent of phototransduction in the vertebrate retina where light sensation causes a decrease in cGMP through activation of PDE. By analyzing other *chb* suppressors, we should be able to identify additional components acting downstream of sensory inputs to regulate the growth and behavioral state of *C. elegans*.

Experimental Procedures

Strains and Genetics

Wild-type animals are the *C. elegans* strain N2. Worms were grown at 20°C in a well-fed condition using standard methods (except the egg-laying assay [25°C] and dauer-formation assay [27°C]) (Brenner, 1974). In the liquid culture condition, ~50 newly hatched L1 stage animals were transferred to a single well of 24 well-plates containing 250 μl M9 solution dissolved with ~10 μl of a bacterial (OP50) pellet. An OP50 aliquot was provided every 3 days.

Strains used in this work included *osm-6(p811), che-2(e1033), che-3(e1124), osm-3(p802, n1545), tax-2(p671), tax-4(p678),* and *egl-8(n488)*. In addition, *egl-4(n478)* was used for complementation tests; *ky27, ky185, n477, n478, n479,* and *n612* were used for *egl-4* mutation site analysis; and *eg36, eg43, eg44,* and *eg64* are new alleles of *egl-4*. *che-2 Ex[EcoT14I/H20::gfp], che-2 Ex[sra-6::che-2/sra-6::gfp]* were provided by Isao Katsura (Fujiwara et al., 1999). *che-2(e1033)* animals were mutagenized with EMS by standard methods (Brenner, 1974), and F2 progenitors with increased body size were identified.

Body Size Measurement

Pictures of animals on culture plates were taken at several time points with a CCD camera (Leica) attached to a dissecting microscope (Leica MZ6). We used Open Lab ver.2.2.5 software (Improvision) to analyze these images and measure the length of the perimeter of the lateral image of animals by tracing them by hand on a monitor (137× magnification). The body size measurement at each time point represented the average perimeter of at least 20 animals. All strains on the same graph were measured at the same time. We did the same time-course experiment independently at least three times. Although we observed a slight variation of body size from experiment to experiment, the ratio of mutant body size to wild-type animal was essentially same.

For the classification of *chb* suppressors into class I and class II, the *che-2* gene was introduced into each *chb;che-2* double mutant as an extrachromosomal array instead of crossing out the *che-2* mutation. Animals with and without this array were compared for body size. *egl-4(eg64)* single mutants made by crossing out the *che-2* mutation exhibit essentially the same body size as *egl-4(eg64); che-2(e1033) Ex[che-2]*.

Behavioral Analysis

Tracking assay: single, well-fed L4 stage animals were transferred to individual 3.5 cm seeded NGM plates and allowed to move freely for 17–20 hr at room temperature. We analyzed at least 20 animals for each strain and repeated the experiments on at least two different days.

Frequency of body bends: The assay was done as described by Koelle and Horvitz (1996). We counted the body bends of worms moving forward continuously on an undisturbed plate or immediately after prodding with a platinum pick.

DIAS analysis: single animals (16–20 hr after the L4 stage at 20°C) were placed on individual 3.5 cm seeded plates. The movement of each single animal was recorded using Open Lab ver.2.2.5 software (Improvision) via the CCD camera (Leica) attached to a dissecting microscope (at 5×) (Leica MZ6). Recording was programmed to start 30 min after positioning of the plate and continued for 100 min (1 frame every 5 s). The image of the movement of an animal was digitized by DIAS ver.3.1 software (Solltech) to enable computation of speed and direction changes. Speed was calculated from the change of the position (centroid of an animal) between two frames. Direction changes were calculated from the change of the positions through three frames. If the direction change was greater than 180°, it was subtracted from 360°, giving values between 0° and 180°.

Segmentation Procedure: the segmentation procedure utilized had previously been used by Pierce-Shimomura et al. (1999) to identify periods of turning of *C. elegans* when animals are off of food. In the absence of food, bursts of turning (pirouettes) can be distinguished from periods of smooth motion (running). It is unclear whether states of roaming and dwelling exist off food (animals move more continuously off food, and animals showing periods of extended slowing during a recording were excluded in the above study). See Supplemental Data online at http://www.neuron.org/cgi/content/full/36/6/1091/DC1 for additional details of behavioral assays.

Molecular Biology Methods

che-2 and *egl-4* cDNA expression: for *che-2* expression by the *che-2* promoter, we used the genomic EcoT14I fragment, which contains the whole coding region and upstream region of this gene (Fujiwara et al., 1999). For *che-2* cDNA expression by other promoters, the *che-2* full-length cDNA was obtained from the *sra-6*::*che-2* construct (Fujiwara et al., 1999). For *egl-4* cDNA expression, *egl-4.a* and *egl-4.b* full-length cDNAs with 3′UTR regions were obtained from the 325g6 and 87g12 cDNA clones (provided by Yuji Kohara). Promoter regions were PCR-amplified and ligated in frame with these cDNAs (see Supplemental Data for detail).

egl-4 GFP fusion constructs: for *egl-4.a*::gfp and *egl-4.b*::gfp, pPD95.69 (GFP expression vector provided by Andy Fire) was used. For the *egl-4.a*::gfp::*egl-4.a* construct, pPD103.87 (tagged GFP expression construct provided by Andy Fire) was used (see Supplemental Data for detail).

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